ON THE POSSIBLE RELATIONSHIP OF THE PREDILECTION SITE FOR THE GLIAL ABNORMALITIES IN THE MALFORMED FETAL MOUSE BRAIN TO THAT FOR THE HUMAN INFANT'S GLIOMAS

by

Goro Shirota

Ist Surgical Division, Kyoto University Medical School
(Director : Prof. Dr. Chisato Araki)

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INTRODUCTION

It has been generally recognized through many observations that there seems to be some relation between the occurrence of the infant's gliomas and the malformation of the brain. Cushing (1930, 31) postulated that medulloblastomas and foastrocytomas of the roof of the fourth ventricle might arise from embryonal cell rests. Globus, Kuhlenbeck described in 1942 that subependymal plate gliomas originated from embryonal cell rests of the subendymal plate. These statements seem to support Cohnheim's "stray germ theory" of tumor formation, by which he meant that tumors developed from embryonic cell rests which remained undifferentiated without participating in the formation of normal tissue.

Shimada (1954), one of the members of our laboratory, examined the brains of human fetuses in a chronological manner, to investigate the development and distribution of immature glial cells and the state of the inclusive cell rests, and he stated that "it would be more reasonable to consider that gliomas might arise from normally long persisting, immature cells rather than from heterotopic or heterotaxic cells".

Bruzstowiz and Kernohan concluded, through their extensive and systematic studies on the correlation between the development of gliomas in the region of the fourth ventricle, and the frequent occurrence of cell rests around the fourth ventricle, that there was not always a definite correlation between the existence of cell rests and the occurrence of gliomas.

Even they, however, pointed out the fact that the nodulus of the cerebellum was one of the predilection sites for the gliomas and also it was the place in which the mixed cell rests were most frequently found.

It is well known that some congenital tissue-malformations of the brain as well as those of other organs, develop under external influences in a certain viviparous period. Thus, the specially treated fetal mice, including both the fetuses grossly malformed and those normal in appearance, i. e. the fetuses which were taken out from the body of the mother mice which had been intravenously injected with trypan blue during the period of their pregnancy, were used for the present study.

The development and maturation of glial cells in these brains were carefully compared with those of normal fetal brains. The data obtained from this animal experimentation were further discussed in relation to the predilection of occurrence of the human infant's gliomas.

MATERIALS AND METHODS

A. Materials

Group 1. 19 day normal fetal mice of hybrid strain.

Grouo 2. 19 day fetal mice of hybrid strain with or without gross malformations which had grown in mother mice who had received intravenously injections of trypan blue on the 8th day of pregnancy.

MURAKAMI succeeded in producing various malformations in the fetuses of mice, by injecting trypan blue to their mothers during pregnancy. He found that the changes in the central nervous system were most severe in fetuses whose mother had been injected with trypan blue on the 8th day of pregnancy.

Following his method, 0.1cc of 0.5% trypan blue solution was injected into the tail vein of each pregnant female animal on the 8th day of pregnancy. On the 19th day of pregnancy, which was a day before the expected day of delivery, the fetuses were taken out from their mothers by laparotomy. Of these fetuses some revealed marked abnormalities in the central nervous system such as pseudencephaly (Fig. 1) or dysrraphy of the brain (Fig. 2), and some others had subcutaneous hemorrhages in the extremities, and still some others were normal at least in

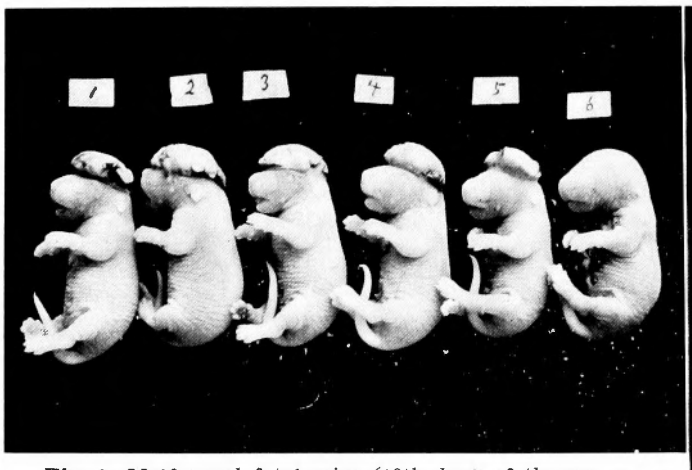

Fig. 1. Malformed fetal mice (19th day) of the same litter whose mother had been injected with 0.1cc of the 0.5% trypan blue solution. Five of six fetuses has pseudencephaly, one is normal in appearance.

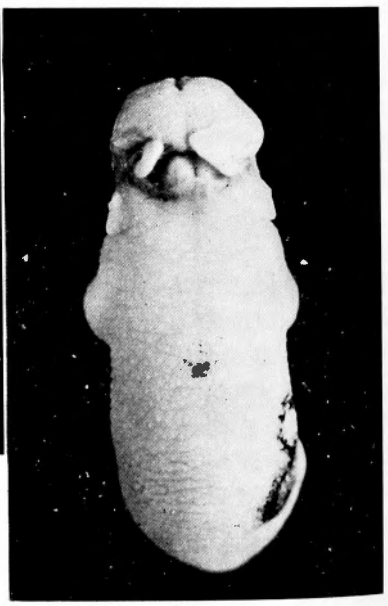

Fig. 2. Dysrraphy of the brain in the pseudencephalic mouse (19th day).

appearance. For the purpose of our study, it is more desirable to use the young mice which had been treated with trypan blue during their intrauterine period, and which were delivered in a normal manner, and which were kept for a certain period after delivery. However, in the present study, we were compelled to use 19 day fetal mice, as described above, because the mothers were apt to eat their malformed young soon after delivery.

B. Methods

Staining

i) PENFIELD'S silver carbonate method, modification II.

ii) RYDBERG'S silver diamino-carbonate method.

Fetal mice were taken out of their mothers by laparotomy. To facilitate the fixation and embedding, the head of the fetus was severed, and washed several times with a physiological saline solution. Fetuses were then fixed in a 10% neutral formalin solution for ten days. After fixation, the head of the fetus was soaked in distilled water for approximately 24 hours. After this, the excess of water was absorbed from the surfaces of the head with blotting paper. Then, the specimen was infiltrated with a 10%, and later 25% gelatine solution, dissolved in a 1% carbol-solution. When an adequate permeation of gelatine into the tissue took place, the gelatine solution was solidified by adding a 10% neutral formalin solution. These gelatine embedded specimens were then frozen and serial sections 15μ in thickness were made. Each section was stained using the aforementioned staining method.

RESULTS

A. Distribution and Development of Glial Cells in the Brain of Normal 19 Day Old Fetal Mice.

1. Rhombencephalon

Cerebellum :

Beneath the pia mater, a comparatively large number of apolar cells, forming the external granular layer, were found.

The molecular layer was rather narrow, and some round or oval apolar cells which seemed to be migrating toward the internal granular layer from the external granular layer were observed. This finding was most obvious in the inferior vermis, especially, around the cerebellar nodulus. PURKINJE'S layer had not yet developed. In the internal granular layer, the predominant cells were apolar neuroblasts, and many argentophile apolar spongioblasts were scattered among them. In the white matter, the polar spongioblasts and polar neuroblasts arranged themselves perpendicularly to the surface. In the caudal part of the roof of the fourth ventricle, namely in the cerebellar nodulus, there remained many immature apolar cells (Fig. 3). Some of them were arranged along the surface of the cerebellum merging into the external granular layer.

Anterior medullary velum :

In this flat and narrow part, apolar spongioblasts and apolar neuroblasts were

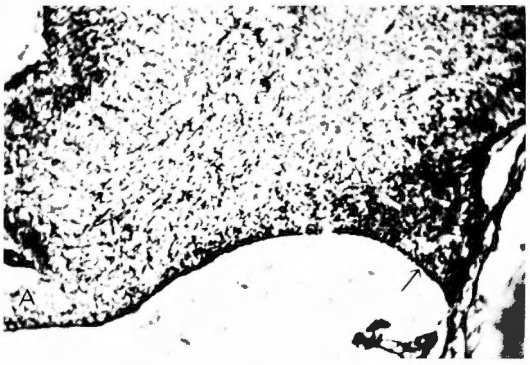

Fig. 3. Midline sagittal section of the normal fetal mouse cerebellum (19th day). Many apolar cells are seen in the region of cerebellar nodulus (indicated by arrow).
A : anterior medullary velum. Penfield's stain Ⅱ.
×100.

Fig. 4. Frotnal section of the normal fetal mouse brain (19th day), showing many apolar cells in the region of taenia rhombencephali(ponticulus)as indicated by arrow. Ⅳ. Ⅴ : fourth ventricle.
F : plexus chorioideus. C : cerebellum.
Normal fetal mouse brain(19th day). Frontal section. Penfield's stain Ⅱ.
×100.

chiefly seen. The matrix was very thin.

Medulla oblongata :

The matrix in the subependymal layer around the fourth ventricle was generally thin, though it was somewhat thicker at the lateral walls than at the floor. In a rather restricted area around the median sulcus there were found comparatively abundant immature apolar cells (Fig. 24). Many nuclei of the crainal nerves were situated near the floor of the fourth ventricle, and around these, immature cells, consisting of apolar spongioblasts and apolar neuroblasts were more abundantly seen in comparision with other parts of the medulla oblongata. Many of these immature cells were also seen in the taenia rhombencephali (ponticulus) (Fig. 4). Coming ventrally, the maturity of the cells gradually increased, and there were many polar spongioblasts and polar neuroblasts. In parts, even astroblasts and astrocytes began to appear, and these were seen most abundantly at the base of the medulla oblongata.

Pons :

In the ventral part of the pars basalis pontis, bipolar spongioblasts and polar neuroblasts were fairly numerous and packed closely together. In the brachium pontis, there were found astroblasts and astrocyte-like cells distributed along the course of the fibers. The dorsal part of the pars basalis pontis was rather acellular and few piloid astrocytes, astroblasts and bipolar spongioblasts were scattered among the interlacing fibers of the pons (Fig. 5). Along the raphe of the tegmentum, i. e. around the nucleus dorsalis raphes and the nucleus centralis superior, densely packed cell groups which were composed of apolar cells and some unipolar and bipolar spongioblasts, were observed. Laterally to these areas towards the brachium conjunctivum, the cell distribution became less marked. Astroblasts and astrocytes were

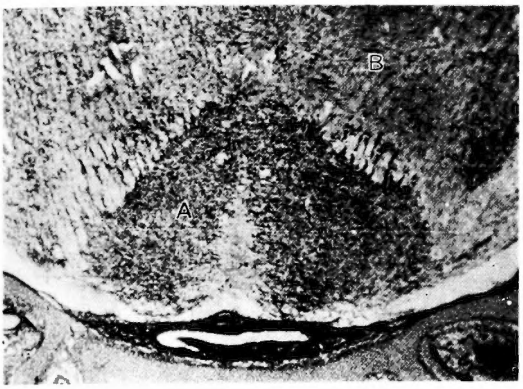

Fig. 5. Frontal section of the normal fetal mouse brain (19th day), showing (A) immature polar cells in the ventral part and, (B) piloid astrocytes, astroblasts and bipolar spongioblasts in the dorsal part of pons. Penfield's stain Ⅱ. ×100.

Fig. 6. Closely packed immature cells in inferior colliculus (indicated by arrow). A: anterior medullary velum. C: cerebellum. Normal fetal mouse brain(19th day). Sagittal section. Penfield's stain Ⅱ. ×100.

the chief cellular elements in this part.

2. Mesencephalon

Quadrigeminal body:

At this stage of the embryonal development (19 day fetus), there were found a good many immature cells in the quadrigeminal body. Around the inferior colliculus, particularly, very densely packed apolar cells were observed (Fig. 6). Approaching the superior colliculus, the density of the glial cells gradually decreased, while polar spongioblasts and polar neuroblasts increased in number.

Pars tegmentum mesencephali:

In the roof of the aqueduct, the matrix layer was fairly thick near the midline and it became thinner laterally. Many apolar cells and immature polar spongioblasts were found in the central gray.

Around the red nucleus and oculomotor nuclei the cells were rather few in number and astrocytes and astroblasts were the main cellular constituents of these areas (Fig. 7). In the vicinity of the substantia nigra, there were numerous polar spongioblasts and astroblasts, also polar neuroblasts were diffusely intermingled with them.

Pedunculus cerebri:

A few mature glial cells, arranging themselves along the course of fiber bundles at the subpial portion of the pes pedunculi, were found.

3. Prosencephalon

Hypothalamus:

The subependymal matrix around the third ventricle was generally thin except in the ventral portion where many apolar cells were closely packed together making a thick layer. Abundant apolar spongioblasts and neuroblasts were also found in

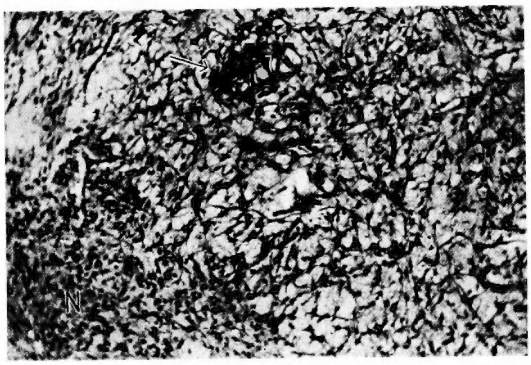

Fig. 7. Astroblasts and astrocytes around nucleus ruber (indicated by arrow). N : substantia nigra. Normal fetal mouse brain (19th day). Frontal section. Penfield's stain Ⅱ. ×100.

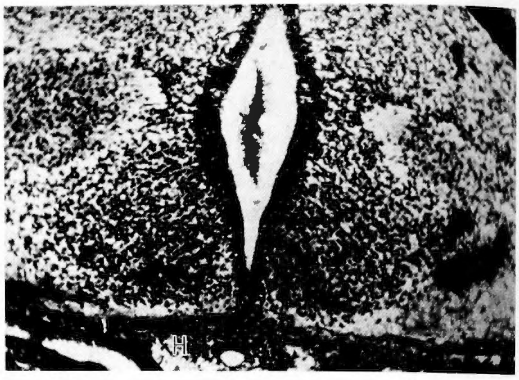

Fig. 8. Frontal section at the level of corpus mammillare. Periventricular matrix around the third ventricle is still thick and densely packed apolar cells are seen in the ventral parts. H : hypophysis. Normal fetal mouse brain (19th days). Penfield's stain Ⅱ. ×100.

the corpus mammillare, recessus infundibuli and in the dorsal half of the optic chiasm facing the third ventricle (Fig. 8). In the remaining part of the hypothalamus polar spongioblasts and polar neuroblasts chiefly were found, while in the lateral portion, i.e. close to the internal capsule astroblasts and astrocyte-like cells were also seen. In the optic chiasm and the optic tract, piloid astrocytes, astroblasts and astrocytes were observed extending their protoplasmic processes in a parallel direction with the nerve fibers.

Thalamencephalon :

In almost all parts of the thalamencephalon the polar cell was the chief cellular element, while in a restricted area of the subependymal layer of the third ventricle, few apolar cells were observed. Also more mature cells such as astroblasts and astrocytes were found subpially in the vicinity of the pulvinar.

Telencephalon :

The matrix layer around the lateral ventricle generally was far thicker than that around the third ventricle. And around the frontal portion of the lateral ventricle, it was thicker than that around the occipital portion of the same ventricle. In these matrices immature apolar cells were seen, but towards the intermediary zone, polar cells began to appear. In the intermediary zone unipolar and bipolar spongioblasts which probably had migrated from the subependymal layer, were very regularly arranged (Fig. 9). Near the cortex, however, this regular arrangement was lost and astrocytes or occasionally astroblasts appeared. In the "Ganglienhügel" which is a part of the caudate nucleus facing the lateral ventricle, groups of immature apolar cells were noticed (Fig. 10). Laterally to the "Ganglienhügel" polar spongioblasts were seen and these seemed to be migrating into the globus and putamen passing through the internal capsule. In the cortical layer, apolar neuroblasts were abundant. At the outermost part of the cortex, i. e. right beneath the pia mater polar and apolar spongioblasts showed a parallel arrangement along the

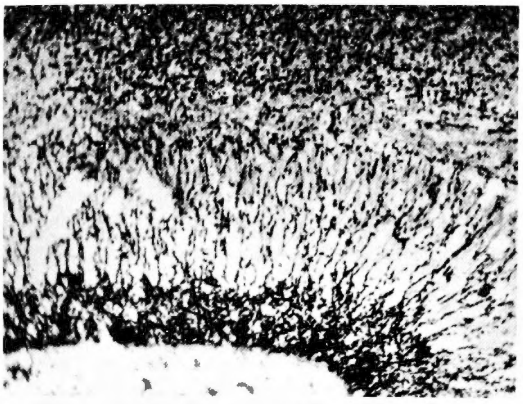

Fig. 9. Sagittal section of the normal fetal mouse brain (19th day), showing the polar cells, migrating from the subependymal layer of the lateral ventricle to the intermediary zone. Penfield's stain Ⅱ. ×100.

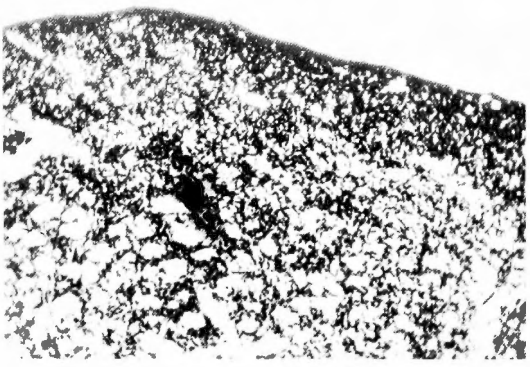

Fig. 10. Sagittal section of the normal fetal mouse brain (19th day), showing a dense immature apolar cell nest in the region of "Ganglienhügel". Penfield's stain Ⅱ. ×100.

pia. Piloid astrocytes and astroblasts were found in the anterior commissure. At the corpus callosum, there were found polar spongioblasts, piloid astrocytes, astroblasts and probably oligodendroglias, arranged in a direction parallel to the fiber bundles. A great many apolar cells were found in the subependymal layer of the septum pellucidum, and coming medially, polar spongioblasts appeared. At the border of the corpus callosum, astroblasts and astrocytes were observed. The cavum septi pellucidi had not developed yet at this stage. In the fornix, protoplasmic and piloid astrocytes were the predominant cells and a few astroblasts were also seen.

B. Distribution and Development of Glial Cells in the Brain of Fetal Mice Treated with Trypan Blue during the Intrauterine Period.

As described before, fetal mice whose mothers had been treated with intravenous injection of trypan blue were further divided into two groups. The first group was composed of the fetal mice with marked gross changes such as pseudencephaly or subcutaneous hemorrhage of the four extremities, and the second was a group of at least macroscopically normal appearing fetuses.

Ⅰ. Findings of the brain of the fetal mice which revealed gross malformations in the central nervous system.

In the present study, pseudencephaly and dysrraphy of the brain were the only gross malformations which we could observe in the central nervous system. The *"distention" of the central nervous system, which was one form of malformations demonstrated in the MURAKAMI's experiment, was not seen in the fetal mice throughout the whole course of our study. This was probably due to the circumstance that we did not take the fetuses out until later i. e. a day before the term of their

* "Distention" is another type of malformation consisting of a hypertrophy and swelling of the central nervous system especially of the brain and an irregular zigzag course of the medullary tube axis (SNELL 1934).

expected delivery. MURAKAMI also stated that fetuses with this malformation usually died early in the course of pregnancy. Actually, in our study, we often observed dead fetuses which had malformations suggestive of the "distention" of the central nervous sytem. 10 cases of pseudencephaly or dysrraphy of the brain were available for histological study. As demonstrated by MURAKAMI pseudencephaly was always associated with dysrraphy of the brain. The roof of the cerebellum and the mesencephalon was separated at the midline, and everted outwards, exposing the floor of the fourth ventricle, aqueduct and the choroid plexus of the fourth ventricle. The telencephalon was also exposed and an asymmetry of both hemispheres was seen. Serial sagittal (Fig. 11) and frontal (Fig. 12) sections were made in these brains and examined histologically.

Fig. 11. Midline sagittal section of the fetal mouse brain of pseudencephaly (19th day). Di : diencephalon. Me : mesencephalon.
P : pons. M : medulla oblongata. Ⅳ.Ⅴ: fourth ventricle. III.V : third ventricle. H : hypophysis. Penfield's stain Ⅱ.

Fig. 12. Frontal section at the diencephalon of the pseudencephalic fetal mouse brain (19th day). Di : diencephalon. III.V : third ventricle. Te : telencephlon. Penfield's stain Ⅱ.

1. Rhombencephalon

Cerebellum :

Marked dysrraphy was noticed in this part of the brain and the vermis was divided at the midline.

The apolar cells were arranged in the external granular layer. Polar cells arranged themselves towards the inner molecular layer. PURKINJE's layer had not developed yet at this time as is the case in normal fetuses. In the internal granular layer there were found immature apolar cells, and in some cases a tremendous number of these cells could been seen in this area. In the cerebellar white matter, polar spongioblasts and neuroblasts were seen chiefly, but occasionally groups of immature polar cells, apolar spongioblasts and apolar neuroblasts were also found with in it. The undifferentiated apolar cells in the caudal part of the vermis, were greater in number in this group of animals as compared to those in normal fetusus, and this was especially true of the nodulus (Fig. 13 & 14).

Medulla oblongata :

In the subependymal layer at the floor of the fourth ventricle which had been exposed to the outside, there were a few apolar cells and many astrocytes, while many polar spongioblasts and neuroblasts and a considerable number of astrocytes and astroblasts were found in the deeper portion. Astrocytes were the predominant cell element in the taenia rhombencephali. Apolar cells were still of servable around the taenia rhombencephali.

Pons :

Right beneath the pia mater at the ventral potion of the pons, cell groups which were composed of apolar cells were found. A little bit dorsally from this portion astroblasts and astrocytes appeared, and polar cells, astroblasts and neuroblastic cells were intermingled together in the more dorsal parts. In the subependymal part of the exposed fourth ventricle, astroblasts and astrocytes were chiefly seen. Mature astrocytes were mainly found in the brachium pontis.

2. Mesencephalon

Abnormal backward bulging of the mesencephalon was a characteristic feature in pseudencephaly. Dysrraphy was also seen in the quadrigeminal plate.

In the subependymal plate of the floor of the aqueduct and also at the caudal end of the inferior colliculus, many apolar cells were to be seen, while in the tegmentum mesencephali and around the superior colliculus, astroblasts and fully matured astrocytes were abundantly seen and a tendency toward tissue sclerosis was noticed (Fig. 15). In some cases, the abnormal bulging of the mesencephalon was so marked that a secondary degeneration of the tissue took place and cellular element in these areas totally disappeared.

3. Prosencephalon

Diencephalon :

In all of the cases, the third ventricle was fairly irregular in shape because of

Fig. 13. Sagittal section of the pseudencephalic fetal mouse cerebellum (19th day). E : external granular layer. I : internal granular layer. M : mixed-cell rests in folial white matter of cerebellum. N : cerebellar nodulus. Penfield's stain II. ×40.

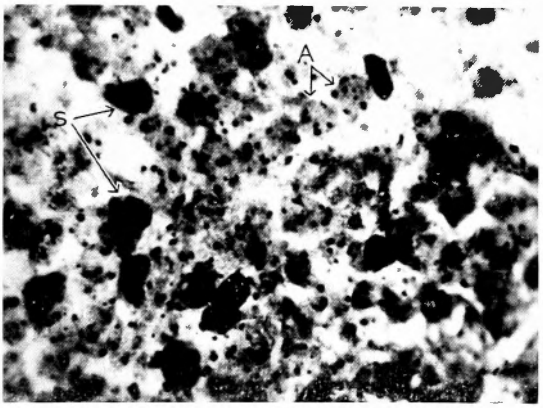

Fig. 14. Higher magnification of part N in Fig. 13. A. undifferentiated apolar cell. S : apolar spongioblast. ×900.

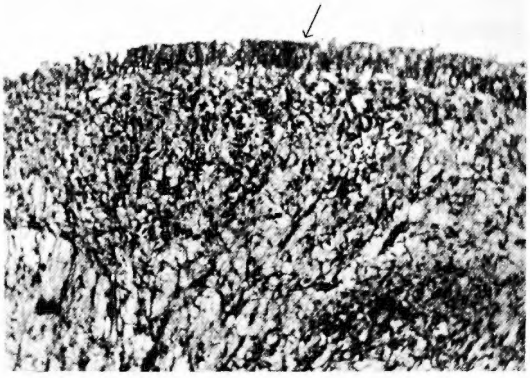

Fig. 15. Sagittal section of the pons and tegmentum mesencephali of the fetal mouse of pseudencephaly (19th day), showing many astrocytes and astroblasts. Arrowy indicates the aqueductus mesencephali. Penfield's stain Ⅱ. ×100.

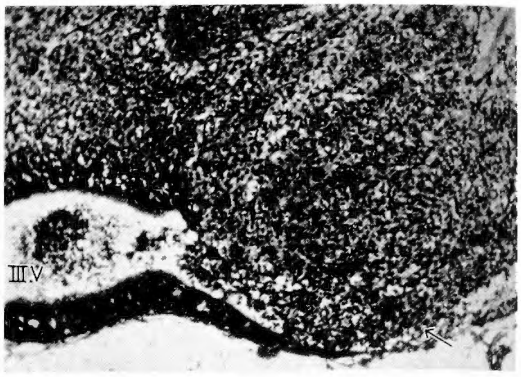

Fig. 16. Dense immature apolar cell nests around corpus mammillare of the pseudencephalic fetal mouse brain (19th day)(arrow indication). III. V : third ventricle. Sagittal section. Penfield's stain Ⅱ. ×100.

the abnormal folding of its wall, and probably also because of the abnormalities of the neighboring nervous tissue such as abnormal bulging of the mesencephalon or diencephalon. Subependymal matrix was generally thin in the third ventricle, but it was somewhat thick in its ventral portion and contained apolar cells. Especially, around the mammillary body, a good many immature cells which were closely packed together in groups, were noticed (Fig. 16), and dorsally toward the thalamus, many polar spongioblasts were seen.

In the thalamus, and at the border to the hypothalamus, there were found mainly bipolar spongioblasts and astroblasts, while in the dorsal part, i. e. approaching the exposed surface, astroblasts and astrocytes increased in number, showing an appearance of gliosis (Fig. 17).

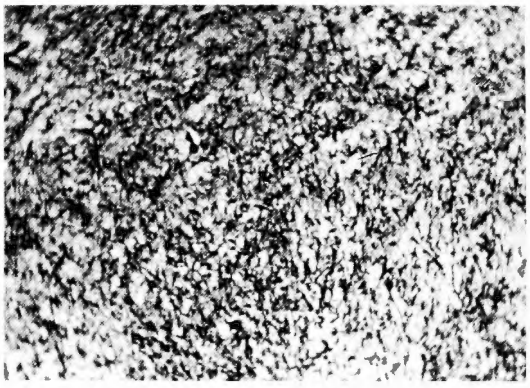

Fig. 17. Sagittal section of the pseudencephalic fetal mouse brain, showing gliosis in the viciniy of the exposed thalamus (19th day). Penfield's stain Ⅱ. ×100.

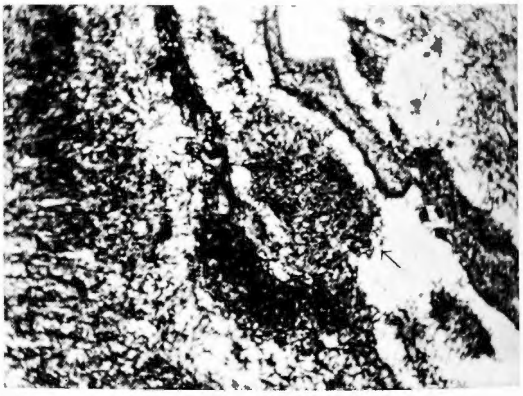

Fig. 18. Closely packed immature apolar cells in the subependymal layer is bulging into the lateral ventricle (indicated by arrow). Pseudencephalic fetal mouse brain (19th day). Frontal section. Penfield's stain Ⅱ. ×100.

Telencephalon :

In pseudencephaly, an asymmetry of the two hemispheres of the telencephalon, though it varied in degree from one to another, was the constant feature. In some cases, the telencephalon was totally exposed, while in the others, it was partly covered by the skin. Changes in the wall of the lateral ventricle were most conspicuous in all cases. Due to the abnormal bulging of the whole brain, the lateral ventricle wall revealed irregular foldings. Paraventricular matrix layer was thick in the frontal region, as was the case in the normal fetus. The thickness of the matrix, however, was somewhat irregular throughout the brain. The circumscribed proliferation of the matrix was common, and in parts, cell groups composed of immature cells extended beyond the ependymal layer to bulge into the cavity of the lateral ventricle and in other parts, they extended towards the outer surface (Fig. 18).

Ⅱ. Heterotopic cell rests in pseudencephaly.

As to the occurrence of the cell rests around the fourth ventricle, Brzustowiz considered the following mechanisms, "(i) a folding of a cell layer, (ii) fusion of several cell layers, (iii) arrest in the migration of germinal elements from the matrix of the fourth ventricle, or from the external granular layer, and (iv) a disturbance or derangement of the normal process of differentiation of germinal cells".

According to this concept, in pseudencephaly, in which abnormal bulging or abnormal fold formation of the brain had taken place during the process of development, the possibility of the occurrence of cell rests should be very high.

In fact, in all cases, many heterotopic cell rests were found in various parts of the brain, and, most abundantly around the fourth ventricle.

It has long been recognized by Pfleger, Brzustowiz and others, that even in the normal human brain, heterotopic cell groups were frequently found around the fourth ventricle. Hence, it would be easy to understand that these changes were more markedly observed this particular part of the brain in pseudencephaly.

Ependymal cell rests at the floor of the fourth ventricle and around the taenia rhombencephali were most common. The ependyma lining the floor of the fourth ventricle tended to be evaginated into the nervous parenchyma or folded and piled together, and these changes were sometimes hard to distinguish from the true ependymal cell rests, especially with a single slide. With serial sections, however, it was confirmed that there were apparent ependymal cell rests, which were composed of tubular or grouped ependymal cells and with no direct connection with the ependyma lining the fourth ventricle (Fig. 19 & 20).

The next commonest was the mixed cell rests in the cerebellar white matter. These consisted of argentophile polar spongioblasts and polar neuroblasts and apolar cells resembling undifferentiated cells, all of these cells being intermingled together.

Occasionally these mixed cell rests were scattered fairly widely throughout the white matter of the cerebellum (Fig. 13 & 21). Brzustowiz stated that the nodulus was a site of predilection for the mixed cell rests in the human cerebellum. In the

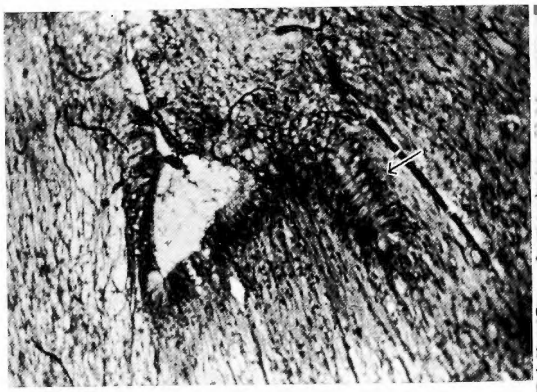

Fig. 19. Evaginated ependymal roll from the floor of fourth ventricle and ependymal cell rests (indicated by arrow). Pseudencephalic fetal mouse brain(19th day). Sagittal section. Penfield's stain Ⅱ. ×100.

Fig. 20. Epenydmal cell rests in the vicinity of the floor of fourth ventricle. Pseudencephalic fetal mouse brain (19th day). Frontal section. Penfield's stain Ⅱ. ×100.

present study, however, we could not find mixed cell rests around the nodulus. Instead, there were many undifferentiated apolar cells. Also in the pons and the tegmentum mesencephali, groups of polar and apolar cells, probably mixed cell rests, were often seen, although these were not observable in the normal fetus. As a rule, glial cells in these parts of the brain in pseudencephaly were quite mature, so the border line between the cell groups of immature cells was fairly clean-cut and easily identifiable (Fig. 22 & 23.)

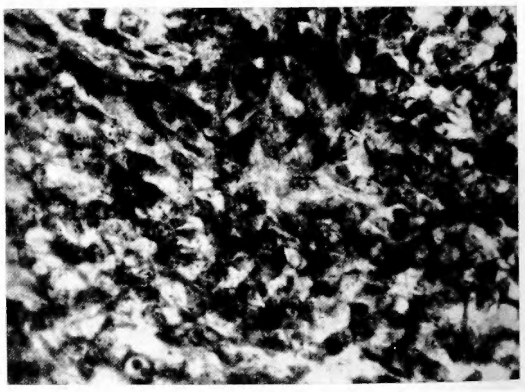

Fig. 21. Higher magnification of part M in Fig. 13, showing cell rests in cerebellar white matter of the pseudencephalic mouse brain (19th day). Penfield's stain Ⅱ. ×400.

Ⅲ. Brain of the fetal mouse which revealed no gross malformation of the central nervous system.

Even in the fetus group which had not revealed any noticeable malformation macroscopically, there were changes or abnormalities in the brain in a fairly high percentage when examined histologically. Provided that some microscopic abnormalities were found in one fetus, they were also observed very often in other fetuses of the same litter though all of these fetuses had not shown any gross malformation.

The microscopic abnormalities were most frequently found in connection with the ventricular system caudal to the mesencephalic aqueduct. Of these, the most

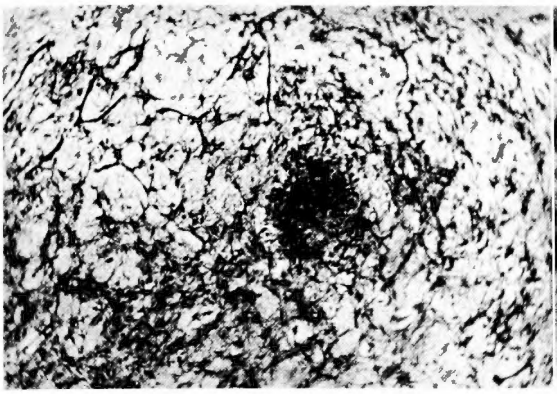

Fig. 22. Group of immature cells, possibly mixed cell rest, in the outer part of the tegmentum mesencephali of the pseudencephalic fetal mouse brain (19th days). Frontal section.
Penfield's stain Ⅱ. ×100.

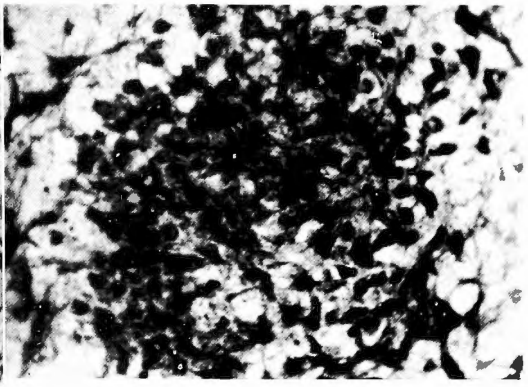

Fig. 23. Higher magnification of Fig. 22. Some polar cells are noticeable.
Penfield's stain Ⅱ. ×400.

striking change was an abnormal accumulation of immature apolar cells in the subependymal layer of the fourth ventricle especially around the median sulcus. Even in normal 19 day fetuses many apolar cells were observed in this area (Fig. 24), but in the abnormal fetus group they were found in a higher degree and over a wider area (Fig. 25). In some cases there were found large numbers of apolar cells remaining in a very restricted area along the median sulcus. In others, argentophilic apolar cells were arranged in line in the subependyma beneath the floor of the

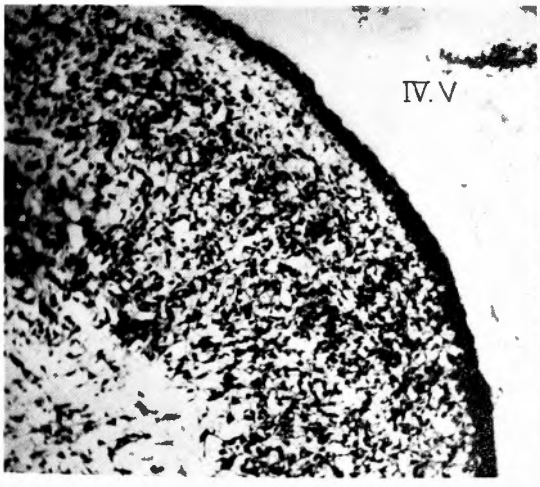

Fig. 24. Apolar cells remain in the subependymal layer at the midline of the fourth ventricle floor. Normal fetal mouse brain (19th day). Sagittal section.
Penfield's stain Ⅱ. ×100.

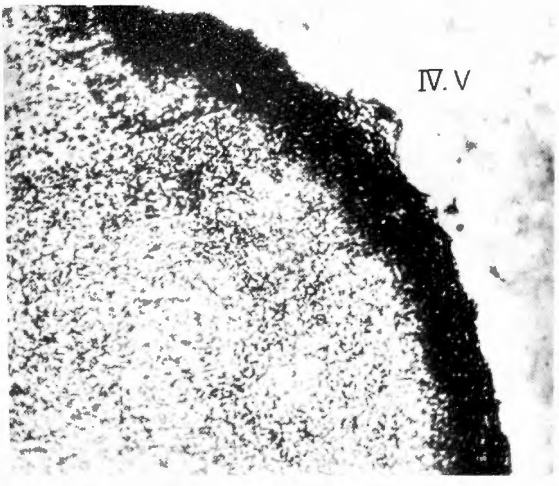

Fig. 25 The same plate with Fig. 24 in the normal appearing fetal mouse brain (19th days), which had been previously injected intravenously with trypan blue. A great many apolar cells were found in the subepependymal layer. Ⅳ. V. fourth ventricle. Sagittal section.
Penfield's stain Ⅱ ×100.

aqueduct or in the deep layer at the floor of the fourth ventricle without connections with the blood vessel (Fig. 26). Deep in the diencephalon, mesencephalon and medulla oblongata, there were found large, round or oval, faintly stained cells forming rosettes (Fig. 27 & 28). WILSON et al. reported that a rosette similar to this was frequently observed in the brain surface of fetal rats which had been irradiated with X-rays on the 9th day of their intrauterine life. In this study, however, we were unable to find the rosettes in the brain surface.

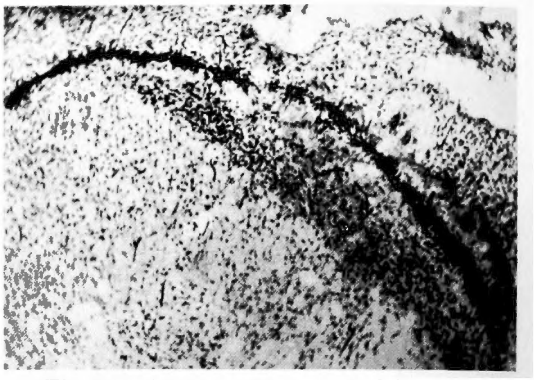

Fig. 26. Apolar cells arranged in row in the deep layer of the fourth ventricle floor. No relation to the blood vessel is observed. Fetal mouse(19th day), treated with trypan blue and with grossly normal appearance. Sagittal section. Penfield's stain Ⅱ. ×100.

Other less common changes were, (i) the subpial accumulation of abundant apolar cells in the vermis, that is, rests of the external granular layer (Fig. 29), (ii) apolar cell groups situated subpially

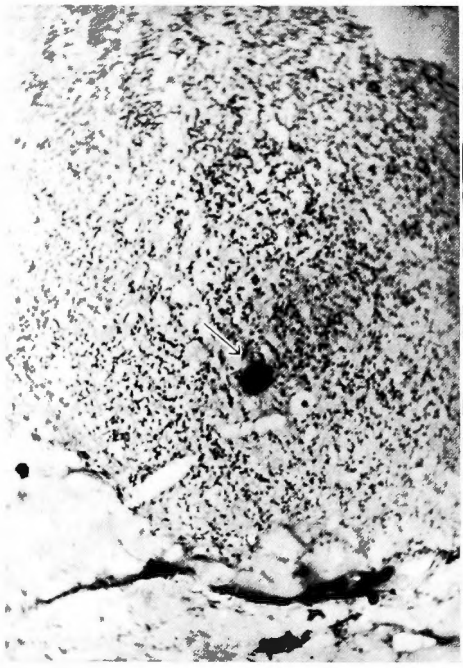

Fig. 27. A rosette formation (indicated by arrow) in the lateral parts of the hypothalamus composed of comparatively large round or oval cells in the fetal mouse brain (19th day) which had been treated with trypan blue but showed grossly normal appearance. Frontal section. Penfield's stain Ⅱ. ×100.

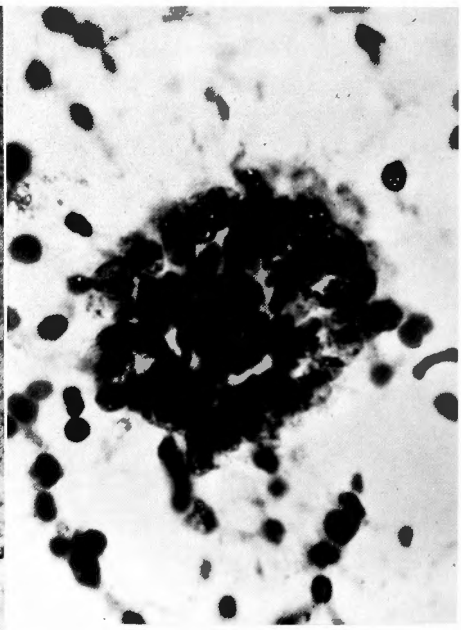

Fig. 28 Higher magnification of Fig. 27. ×900.

at the base of the brain where the olfactory tract emerged (Fig. 30) and (iii) the perivascular accumulation of apolar spongioblasts around the "Ganglienhügel", the last change was also demonstrated in the human fetal brain by SCHWARZ, KERSCHMAN and SHIMADA, and named by ARAKI & SHIMADA as the "perivascular cuff of apolar spongioblast".

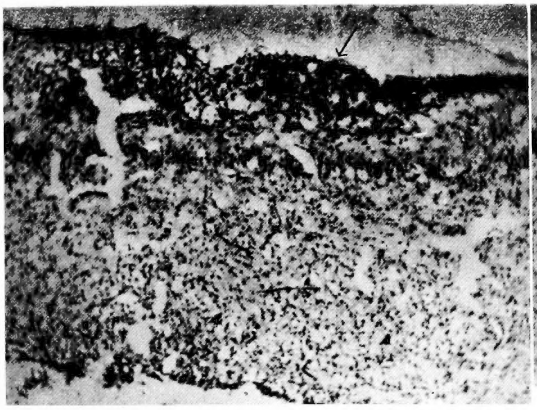

Fig. 29. External granular layer rests in the cerebellar vermis (indicated by arrow) of the trypan blue treated mouse with grossly normal appearance (19th days).
Frontal section. Penfield's stain][. ×100.

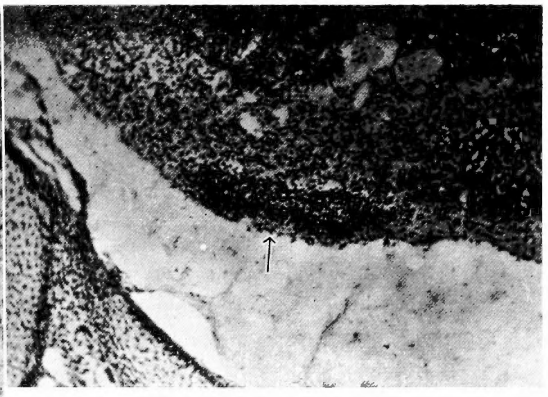

Fig. 30. Subpial apolar cell nest (indicated by arrow) in the basal part of the cereberum of the trypan blue treated fetal mouse with grossly normal appearance (19th day). Frontal section.
Penfield's stain][. ×100.

DISCUSSION AND SUMMARY

Using 19 day fetal mice which had been treated wth trypan blue during their intrauterine period and which were composed of two groups, i.e. (i) grossly malformed and (ii) externally normal fetuses, the distribution and development of glial cells and the state of tissue malformation were examined in various parts of the brain in comparison with those in normal fetuses.

In the normal fetus group, comparatively immature glial cells were noticed at the following sites; taenia rhombencephali, quadrigeminal bodies, especially in the inferior colliculus, base of the pons, ventral part of the diencephalon, around the lateral ventricles especially around the frontal horn, "Ganglienhügel", outer layer of septum pellucidum, and cerebral base. These findings were quite comparable with those of human fetal brains examined by SHIMADA. SHIMADA was of the opinion that there was some correspondence of the sites of predilection for gliomas of infants with anatomical areas where these immature glial cells remained until late in the normal course of embryonal development. In fact, most of the infant's gliomas are likely to occur somewhere in the aforementioned foci of the brain.

In pseudencephaly, which was always associated with dysrraphy, the following changes were noticed. Around the exposed meso-diencephalon gliosis and sclerosis of the tissue took place. In the cerebellar vermis especially around the nodulus numerous undifferentiated apolar cells were found. Mixed cell rests in the cerebellar

white matter, abnormal evagination and folding of the ependyma at the floor of the fourth ventricle, or heterotopic cell rests in the pons and mesencephalic tegmentum were the common features of this malformation. Also, in the ventral part of the hypothalamus many immature cells accumulated, the third ventricle and the lateral ventricles were markedly deformed, and the paraventricular matrix around the lateral ventricles was often extremely proliferated. In the group of fetuses, which had been treated with trypan blue and which revealed no gross malformation in the central nervous system, the following findings were most common. That is, many immature cells existed in the subependymal layer at the floor of the fourth ventricle, apolar cells were arranged in a row in the medulla oblongata, apolar cells formed numerous rosettes in the parenchyma of the diencephalon, and the external granular layer rests were seen in the cerebellum. At the base of the brain a large number of apolar cells were grouped subpially, and the perivascular accumulation of apolar cells was found around the "Ganglienhügel". Some of these findings were not observable in the normal fetuses while others were seen in them. Findings which could be found even in the normal fetuses, however, seemed to be markedly exaggerated and became more obvious in the trypan blue treated fetus group. Summarizing these findings the following conclusion may be drawn. In the trypan blue treated fetus group, either grossly malformed or normal in appearance, immature cells, which were also observable in normal fetal mice, were found more obviously and more abundant by in the same places of the brain as in the normal fetuses. Changes of glial cells in and around the ventricular system and also heterotopic cell rests did most frequently appear caudally to the mesencephalon. Besides these changes, in pseudencephaly, the presence of widely scattered glial ectopic cell rests, the tendency towards retardation of maturity of glia and also the local gliosis around the exposed area of the brain were noticed.

On the basis of this study, it is our impression that the most susceptible places of the mouse brain to the influence of trypan blue, corresponds fairly well with the predilection sites for the infant's glioma. Further, it is interesting to review the data of this study from the standpoint that certain gliomas may possibly originate from embryonal tissue malformation.

Still, we dare not concluded that all gliomas have their origin exclusively in the developmental failure in the brain. Even if there were some relationship between the two, we do not know, as was stated by CUSHING & BAILEY, under what conditions these embryonal cell rests should become neoplastic in character.

CONCLUSION

1) Using normal 19 day old fetal mice and 19 day old malformed fetuses whose mothers had been injected intravenously with a trypan blue solution on the 8th day of their pregnancy, the development and distribution of the glial cells were studied in various parts of the brain. The possible relationship of the sites of tissue abnormalities of the brain the malformed fetuses to the predilection sites for human infant's gliomas was examined.

2) Places where immature cells remained abundantly in the normal fetal brain had a correspondence to the sites of predilection for the human infant's gliomas.

3) In the brain of the malformed animal, there was generally found a tendency to give rise to developmental retardation and ectopia of glial cells, and also many immature cell rests which were not observable in the normal fetal brain. These changes were most frequently observed around the ventriclular system, particulaly caudal to the mesencephalon. These locations again corresponds to those of predilection for the infant's gliomas.

4) Relationship of the embryonal tissue malformation to the occurrence of gliomas is still open to discussion.

However, it might not be unreasonable to consider that under some unknown conditions these tissue malformations will more likely change into gliomas, or at least will be more responsible for the occurrence of infant's gliomas, than the normal brain tissue.

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マウスの異常発育胎仔脳に於ける組織異常の好発部位と人間小児脳グリオームの好発部位との関係に就いて

京都大学医学部外科学第1講座（指導：荒木 千里 教授）
代 田 伍 朗

小児脳グリオームの発生が脳組織異常（cell rests）と関係を有するのではないかと云う点に就ては多くの学者が注目してきたところである．

私は村上の方法に従てトリパン青を妊娠第8日の母マウスに静注し，第19日に開腹によつて取り出した異常発育マウスの胎仔（外見上正常胎仔をも含む）の脳について組織異常の存在を追求し，之が正常なものと如何に異るか，又それの好発部位と小児脳グリオームの好発部位と如何なる関係にあるかを調べ次の結果を得た．

1） 正常19日マウス胎仔に於て此の時代尚比較的未成熟なグリア細胞が残存する部分は小脳の外顆粒層，小脳結節，菱脳紐，四丘体特に下丘，橋脳底，間脳腹側，側脳室周辺，Ganglienhügel 及び大脳底などで，之は人間胎児脳について調べた島田の所見と一致し，小児脳グリオームの好発場所と一部合致している．

2） 妊娠中の親動物にトリパン青を静注して後，妊娠第19日目に開腹して取り出したマウス胎仔に於てはpseudencephaly(dysrraphyを伴う)．四肢の皮下出血の如く一見して異常の存在を認知出来るもの及び外見上何等異常の存在を認知出来ないものとが存在したが，之等胎仔の脳に於ては正常胎仔脳に比して全般としてグリア細胞の ectopia 及び成熟遅延のより著明なる傾向を有し，正常胎仔脳に於て未分化細胞を遺留する部分に於て，同様なる未分化細胞の遺留を正常の場合よりも著明に認めた．又脳室系にも種々な変化が多く認められたが特に中脳以下尾側に多く存在した．此の事からして之等トリパン青の影響を受けたマウスの胎仔脳に於て組織学的変化を生じ易い部分は人間小児脳に於けるグリオームの好発場所と一部合致している如く思われる．